

NOTE TO THE FILE

February 3, 1998

Subject: BNF0054 - Insect Resistant Tomato

Keywords:

Tomato, *Lycopersicon esculentum*, cryIA(c), *Bacillus thuringiensis* subsp. *kurstaki*, kanamycin resistance gene, *kan'*, aminoglycoside 3'-phosphotransferase II (APH(3')II), neomycin phosphotransferase II (nptII), *aad*, aminoglycoside adenyl transferase.

Background

Calgene, a subsidiary of Monsanto, developed the insect-resistant (IR) tomato through insertion of a synthetic version of the cryIA(c) gene encoding a truncated version of the CryIA(c) protein. In a submission dated December 22, 1997, Calgene provided to FDA, a summary of the safety and nutritional assessment it had conducted on its IR tomato.

Intended Effect and Food Use

The intended technical effect of this genetic modification of tomato is to confer resistance to certain lepidopteran insect pests. Calgene stated that the food uses of IR tomatoes are no different than those of traditionally bred tomatoes, i.e., consumption of the fruit as a vegetable eaten raw and in a variety of processed forms.

Molecular Alterations and Characterization

The IR tomato was produced by Agrobacterium-mediated transformation of tomato cultivar UC82B and the resulting transformation event Line 5345 was used as the parental line in a tomato breeding program for development of insect resistant tomato varieties. Calgene stated that the T-DNA was stably integrated in the new tomato variety (line 5345) genome as shown by molecular analysis over seven generations. The IR tomato contains 1) the cryIA(c) gene (codon-optimized for expression in plants) derived from *Bacillus thuringiensis* subsp. *kurstaki* and having the 35 S cauliflower mosaic virus (CaMV) promoter and a 3' nontranslated region of soybean alpha subunit of the beta-conglycinin gene, 2) the *kan'* gene from *E. coli* Tn5 with the CaMV 35S promoter and the NOS 3' nontranslated region for transcription termination and polyadenylation, and 3) the *aad* gene (encoding streptomycin adenyltransferase) under a bacterial promoter that allows for bacterial selection on spectinomycin or streptomycin in the steps prior to plant transformation.

Expressed Protein

Calgene stated that two new proteins are expressed in the IR tomato: a truncated version of the CryIA(c) protein encoded by the *cryIA(c)* gene, and aminoglycoside 3'-phosphotransferase II (APH(3')II) encoded by the *kan'* gene. Calgene noted that while the *aad* gene is present in the tomato genome, it is not expressed because it is under its own bacterial promoter.

Regulatory Considerations

The use of pesticidal substances as well as the use of selectable markers as inert ingredients in the development of pest-resistant plant varieties is under the regulatory purview of the Environmental Protection Agency (EPA). Thus, EPA regulates the use of the CryIA(c) and the selectable marker APH(3')II¹ as well as the genetic material encoding them. Therefore, although Calgene presented information regarding these proteins, including expression levels, we have not addressed the safety of use of these proteins. The main focus of this consultation is on compositional analysis of this transgenic tomato as compared to the parental or other commonly consumed varieties.

Compositional Analysis

Endogenous toxicant

Calgene measured tomatine in mature green and red ripe fruits of IR tomato line 5345 and control tomato plants from three field sites. As expected tomatine levels in fruit from both lines declined as fruit ripened from mature green to red ripe. Calgene reported that the tomatine content of mature green fruit, averaged across field sites, was slightly higher in tomato line 5345 (51.5 µg/g fresh fruit) than in the control line (37.9 µg/g fresh fruit). However, the level of tomatine in mature green fruit of the IR tomatoes was well within, and at the low end of the range of tomatine levels reported in the literature for tomatoes (49-900 µg/g fresh fruit). Calgene attributes this minor difference to the inherent variability of tomatine levels between tomato plants and not due to the genetic modification. Calgene also reported that tomatine levels in red ripe fruit was very low in both IR tomato line 5345 and control lines with no statistical difference seen between the two.

¹The use of APH(3')II in the development of transgenic tomato, cotton, and oilseed rape is approved by FDA as a food additive (21 CFR 173.170; 21 CFR 573.130). The Environmental Protection Agency has established an exemption from the requirement of a tolerance for residues of APH(3')II and the genetic material used to produce it when produced in plants as a plant pesticide inert ingredient (56 FR 49351; September 28, 1994).

Nutrients

Calgene performed studies aimed at comparing the nutritional constituents of tomato fruit from IR tomato line 5345 with those of fruit obtained from control plants grown, processed and analyzed under the same conditions. Parameters measured include total solids, protein, ash, carbohydrates, calories, vitamin A, vitamin C, and folic acid. Values obtained for each parameter measured for tomatoes grown in separate sites were averaged before the comparison was made. Calgene stated that there were no statistically significant differences for any of the parameters between IR tomato fruits and control fruits and that the results of the analysis demonstrate that tomato fruits produced by IR tomato plants are substantially equivalent to those produced by nontransformed controls.

Processing Component: pH

Because the low pH of tomato fruits prevents growth of thermophilic organisms during tomato processing and therefore is an important quality trait from the standpoint of product safety, Calgene measured the pH of red ripe IR tomatoes and control tomatoes. Calgene reported that there was no statistically significant difference in fruit pH between IR and control tomatoes and that fruit pH of both lines was within range of values reported in the literature for tomatoes.

Conclusions

Calgene has concluded that its transgenic tomato line 5345 is not materially different from nontransgenic controls in terms of food safety and nutritional profile. At this time, based on Calgene's description of its data and analyses, the Agency considers Calgene's consultation on tomato line 5345 to be complete.

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